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Fluorescent Intensity of Dye Solutions under Different pH Conditions

ABSTRACT: Fluorescent tracers are widely used for assessment of spray quantity in the field due to their relatively high sensitivity, low cost and user safety. However, many concerns have been raised over their measurement accuracy due to questions of stability of fluorescence during tests. Stable analysis of fluorescence is essential to ensure accurate evaluation of pesticide spray application efficiency. The objective of this research was to determine the stability of fluorescent intensity of five tracers dissolved in solutions with various pH conditions in an effort to minimize analytical errors in the measurement of spray deposition and drift. The fluorescent intensity of five fluorescent tracers commonly used for the quantitative assessment of spray deposition and off-target loss was investigated with wash solutions over pH conditions from 6.9–10.4. The tracers selected in the tests were Brilliant Sulfaflavine (BSF), Fluorescein, Pyranine, Tinopal, and Eosin. The fluorescence of Pyranine was the most sensitive to the solution pH conditions, followed by Fluorescein and Tinopal, while BSF and Eosin had a nearly constant fluorescent intensity over the pH range from 6.9–10.4. The fluorescence of Fluorescein increased 1.3 times, Tinopal 1.25 times, and Pyranine 3.0 times as the pH value increased from 6.9– 8.4, but it became nearly constant when pH value was greater than 8.4. However, Pyranine, Fluorescein, and Tinopal showed much stronger fluorescence than BSF and Eosin. A solution containing Fluorescein at pH 8.4 and higher demonstrated 83 times greater fluorescent intensity than the solution containing the same amount of BSF. In conclusion, the fluorescence of tracers should be examined under various pH conditions during the selection of tracers for pesticide spray deposition and drift trials.

KEYWORDS: fluorescence tracer, spray drift, deposition, wash solution, pesticide

Introduction

Pesticides have ensured high productivity of agriculture and a quality supply of food and fibers in past decades. Agricultural production and storage consumes about 75 % of total pesticide in the USA. However, pesticide use can raise concerns about health risks from residues in food and drinking water, worker hazards, and negative impacts on wildlife and sensitive ecosystems.

To improve pesticide spray application efficiency and to reduce environmental contamination, it is important to measure the spray quality and quantity reaching target and off-target areas. Many field tests have been conducted using fluorescent tracers to measure pesticide spray deposition and drift [8,11,6], evaluate spray penetration and coverage [3,14,7], track spray

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deposits at various locations within canopies [5,15], and improve sprayer design [9]. Fluorescent tracers have advantages of high sensitivity, relatively low cost, and user safety to quantify pesticide spray deposits and drift in the field.

While fluorescent tracers are widely used for assessment of spray quantity, many concerns have been raised over their measurement accuracy due to questions of stability of fluorescence during experiments. There has been considerable research on photo degradation of fluorescent tracers that can result in a severe underestimation of the retained deposit [13,4,16]. The rate of fluorescent degradation under sunlight varies with the type of fluorescent substances [2,10].

The quality of wash solutions used to dissolve tracers for quantitative spray analysis is another element that influences fluorescent measurement accuracy. In field experiments, water is usually used as a carrier to mix with tracers for spray experiments, but the level of minerals and other elements existing in water varies with water sources and locations. For laboratory analysis, distilled water is often used as the wash solution to dissolve spray samples containing tracers. Among various effects, unexpected materials from samples washed into the solution could alter solution pH. The behavior of some fluorescent substances appeared to be different in alkaline and acidic solutions [12]. However, little information is available on the influence of solution pH conditions on the analysis of fluorescent tracers commonly used in evaluation of pesticide spray application efficiency.

The objective of this research was to determine the stability of fluorescent intensity of five tracers dissolved in solutions with various pH conditions in an effort to minimize analytical errors in the measurement of spray deposition and drift.

Materials and Methods

The fluorescent intensity of five different water soluble fluorescent tracers commonly used to quantify agricultural pesticide spray deposition and drift was tested under various wash-solution pH conditions. The five fluorescent tracers were Fluorescein (Aldrich, Milwaukee, WI), Pyranine (Acros Organics of Fisher Scientific, Fair Lawn, NJ), Tinopal (Ciba-Geigy Chemical Corporation, Toms River, NJ), Brilliant Sulfaflavine (BSF) (MP Biomedicals, Inc., Aurora, OH), and Eosin (Acros Organics, Fisher Scientific). The excitation and emission wavelengths, CAS registry number, molecular weight, and chemical formula of these tracers were listed in Table 1. Rhodamine B is another fluorescent tracer often used in quantification of spray deposition, but it was not selected for this work because some formulations contain a suspected carcinogen [1].

TABLE 1—*Fluorescence tracers used in the tests.*

Fluorescence Tracers	Excitation (nm)	Emission (nm)	CAS Registry Number	Molecular Weight	Formula
Fluorescein	494	520	518-47-8	376.3	$C_{20}H_{10}Na_2O_5$
Pyranine	455	508	6358-69-6	524.4	$C_{16}H_7Na_3O_{10}S_3$
Tinopal	350	430	27344-41-8	562.6	$C_{28}H_{20}Na_2O_6S_2$
BSF	460	500	2391-30-2	404.4	$C_{19}H_{13}N_2NaO_5S$
Eosin	525	545	15086-94-9	647.9	$C_{20}H_8Br_4O_5$

The portion of active ingredients varied with the five fluorescent substances. Fluorescein contained 70 % dye content and 30 % sodium salt; Pyranine contained 98 % pyrenetrisulfonic acid trisodium salt known as Solvent Green 7; Tinopal was a bis-benzenesulfonic acid disodium

salt; Brilliant Sulfaflavine and Eosin were the yellowish free acid dyes.

Solution samples were prepared in two steps for fluorescent intensity analysis. The first step was to produce an initial tracer mixture for representing a spray deposition sample collected from the field. The second step was to dissolve the initial tracer mixture in a wash solution with an expected pH value. The initial tracer mixture contained a tracer with either purified distilled water or regular tap water. Purified distilled water was used to make initial mixtures for all tracers, while tap water was used for mixtures only containing Pyranine and Tinopal for an additional trial. The pH value was 6.2 for the purified distilled water and 8.7 for the tap water. The concentration of tracers in the initial tracer mixture was 0.015 mg/mL for Fluorescein, 0.1 mg/mL for Pyranine, 0.0625 mg/mL for Tinopal, 3.0 mg/mL for BSF, and 0.3 mg/mL for Eosin, respectively. The concentrations were selected based on the pre-trials for fluorescent intensity that fell within the detecting range of the spectrometer used in this research.

In the second step of the sample preparation, 10 µl of the initial tracer mixture was dissolved into a wash solution with one of the five pH values (6.9, 7.4, 8.4, 9.2, and 10.4 at 25°C) to obtain final solutions with known tracer concentration. The wash solutions with pH 6.9, 7.4, 9.2, and 10.4 were prepared by mixing distilled water and sodium carbonate (a buffer salt from Fisher Scientific, Fair Lawn, NJ). The solution with pH 8.4 was adjusted from a mixture consisting of 60 % pH 9.0 Fisher buffer solution, 30 % distilled water and 10 % pH 5.0 Fisher buffer solution. The concentrations of tracers in the final solutions were 0.03, 0.015, and 0.0075µg/mL for Fluorescein, 0.05 µg/mL for Pyranine, 0.0315 µg/mL for Tinopal, 0.25, 0.5, 1.0 and 2.5 µg/mL for BSF, and 0.03 and 0.3 µg/mL for Eosin, respectively. For each concentration, three samples were prepared for three replications.

After the final solution with a tracer and a desired pH was achieved, 4 mL of the sample was placed in a cuvette for fluorescence analysis with a Model LS 50B luminescence spectrometer (Perkin-Elmer Limited, Beaconsfield, Buckinghamshire, England).

Data were analyzed by one way ANOVA, and differences among means were determined with Duncan's New Multiple-Range Test using ProStat version 3.5 for windows (Poly Software International, Inc., Pearl River, NY). All significant differences were determined at the 0.05 level of significance.

Results and Discussion

Figure 1 shows the effect of solution pH value ranging from 6.9–10.4 on the fluorescent intensity of Fluorescein at the concentrations of 0.03, 0.015, and 0.0075 µg/mL, respectively. The fluorescent intensity increased as the pH value increased from 6.9 to 8.4, and then became nearly constant when pH value was 8.4 or greater. For example, the average fluorescent intensities of the Fluorescein solutions at 0.03 µg/mL concentration were 671, 767, 861, 867, and 870 with pH values of 6.9, 7.10, 8.4, 9.2, and 10.4, respectively. The fluorescent intensity increased 1.3 times for all three concentration solutions when the solution pH increased from 6.9 to 8.4 or higher.

Similar to Fluorescein, the fluorescent intensity of both Tinopal and Pyranine substances increased as the solution pH value increased from 6.9–8.4, while the fluorescent intensity became nearly constant when the pH value was greater than 8.4 (Fig. 2). The fluorescent intensity was increased 3.0 times for the 0.05 µg/mL Pyranine solution and 1.2 times for the 0.0315 µg/mL Tinopal solution when solution pH increased from 6.9 to 8.4. When solution pH value was greater than 8.4, the mean fluorescent intensity was 634 with 2.7 % coefficient of variation for Pyranine and was 839 with 1.6 % coefficient of variation for Tinopal. Therefore, the

fluorescence for the three substances became weaker when solution was more acidic and became more intense when the solution became more alkaline.

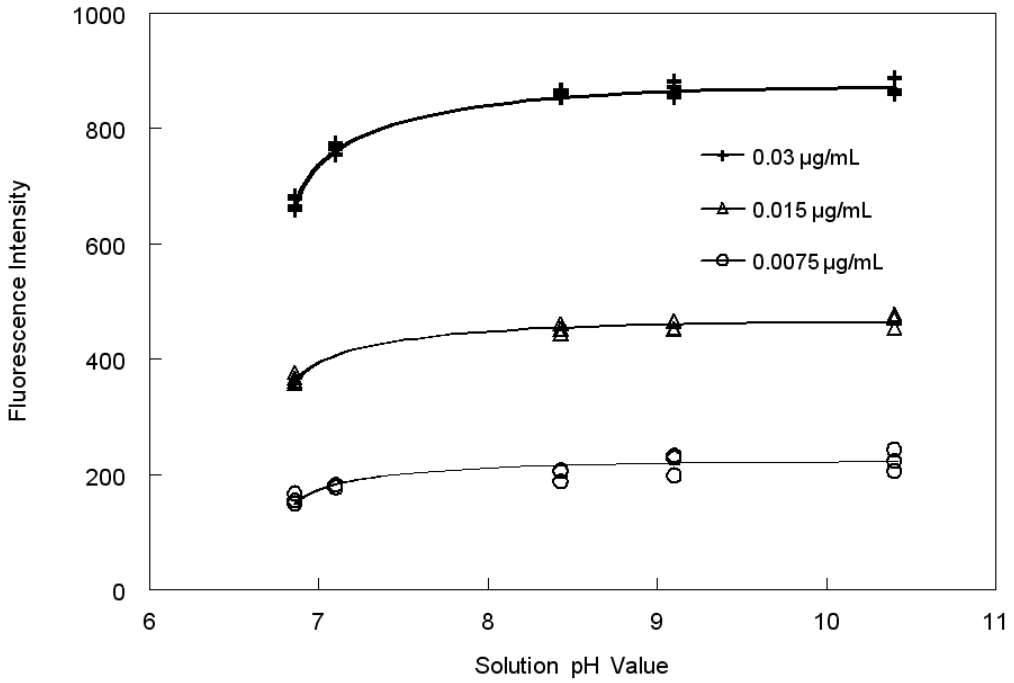

FIG. 1—*Effect of final solution pH conditions on fluorescent intensity of Fluorescein tracer at various concentrations.*

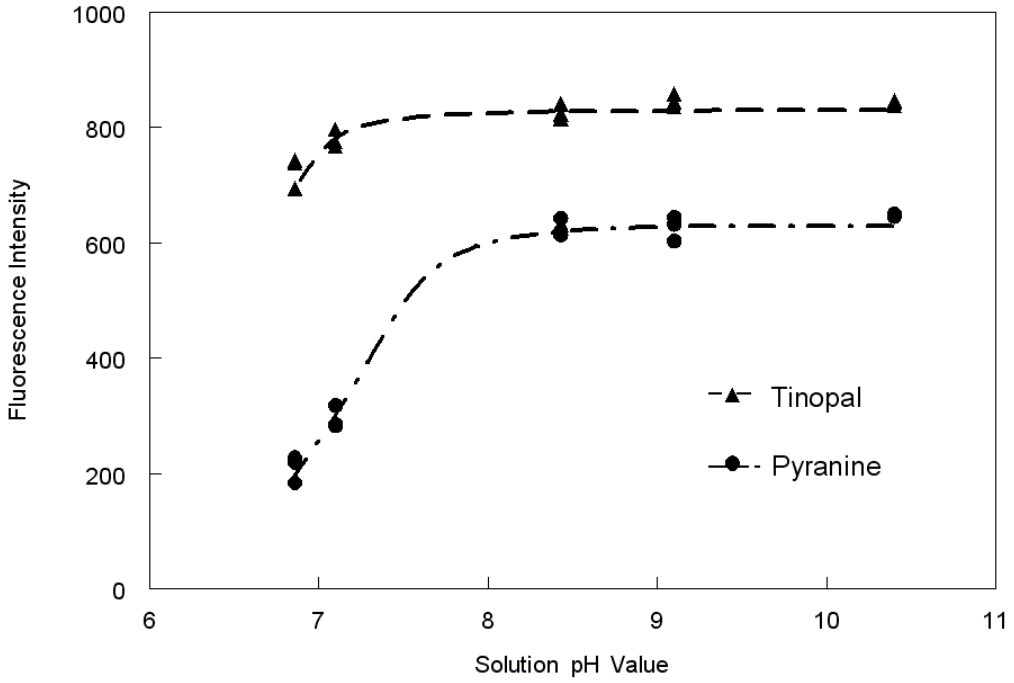

FIG. 2—*Effect of final solution pH conditions on fluorescent intensities of solutions containing 0.0315 µg/mL Tinopal and 0.05 µg/mL Pyranine, respectively.*

The fluorescence of Pyranine, Flourescein, and Tinopal in solutions with different pH conditions varied with the states of ionization formed in either alkaline or acidified solutions. The density of ions in solutions might strengthen the absorbing power for the three tracers. Many previous studies reported that purified distilled water was used to dissolve spray samples containing fluorescent tracers. However, the purified distilled water always has pH lower than 7 because of dissolved CO_2, appearing as an acidified solution. In this state, fluorescent intensity of these three tracers was sensitive to changes in pH conditions (Figs. 1 and 2). To minimize analytical error, a wash solution with a pH value above 8.4 should be used.

The fluorescent intensity of BSF and Eosin had little variation with the solution pH conditions. Figure 3 illustrates that BSF and Eosin at two different concentrations had a nearly constant fluorescence over the pH range from 6.9–10.4. The mean fluorescent intensity was 871, 96, 340, and 180 with 1.3 %, 14.7 %, 4.0 %, and 2.8 % coefficient of variation for Eosin at 0.3 µg/mL, Eosin at 0.03 µg/mL, BSF at 1.0 µg/mL, and BSF at 0.5 µg/mL, respectively. The fluorescent intensity of BSF and Eosin was not affected by solution pH conditions. The fluorescent intensity of different dyes responded differently to solution pH conditions.

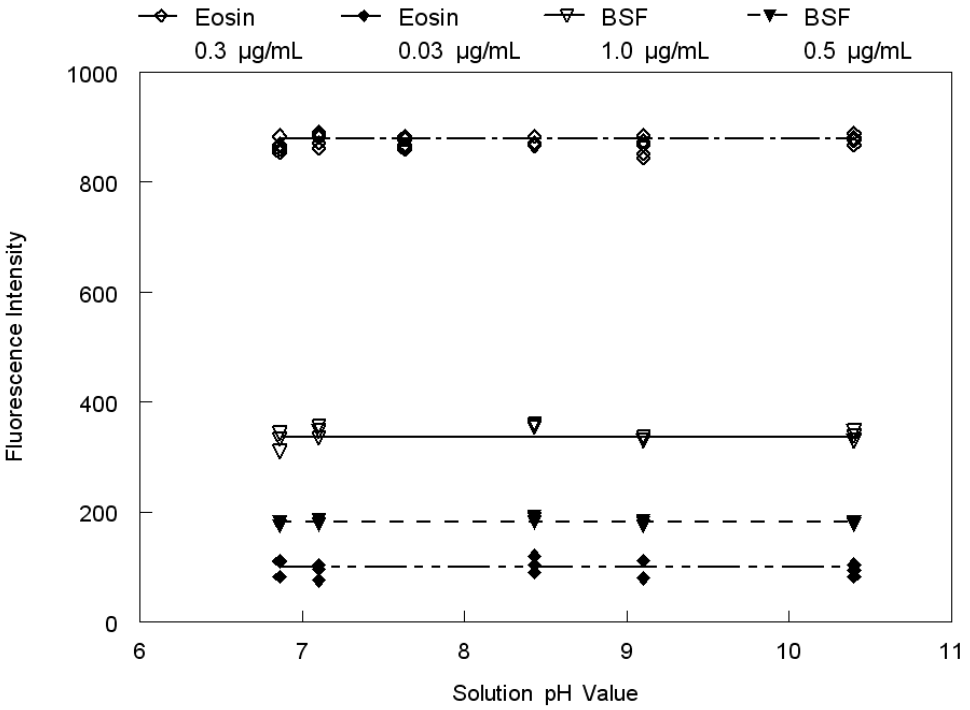

FIG. 3—*Effect of final solution pH conditions on fluorescent intensities of solutions containing 0.03 and 0.3 µg/mL Eosin and 0.5 and 1.0 µg/mL BSF, respectively.*

Table 2 shows the fluorescent intensity of the final wash solutions containing 0.0315 µg/mL Tinopal and 0.05 µg/mL Pyranine with the initial tracer solution containing either distilled water or tap water, respectively. The percentage of the initial tracer solution in the final wash solutions was 0.05. Under the same pH conditions, there was no significant difference ($p<0.05$) in fluorescent intensity for Tinopal solutions using either tap water or distilled water in the initial tracer solution. However, when the pH of the wash solution was higher than 7.4, the Pyranine mixture made with tap water had slightly greater fluorescent intensity than the mixture containing distilled water.

TABLE 2—*Fluorescent intensities of final tracer solutions containing either 0.0315 µg/mL Tinopal or 0.05 µg/mL Pyranine when the initial tracer solution was formed by a tracer with either distilled or tap water. Standard deviations were given in parentheses.*

Final Solution pH value	Fluorescent Intensity			
	Tinopal (0.0315 µg/mL)		Pyranine (0.05 µg/mL)	
	Distilled Water	Tap Water	Distilled Water	Tap Water
6.9	725 (26.8)	741 (4.7)	210 (23.2)	211 (19.6)
7.4	802 (36.6)	780 (50.1)	296 (19.6)	335 (5.6)
8.4	805 (25.7)	804 (31.4)	625 (15.2)	680 (10.6)
9.2	846 (11.7)	824 (27.7)	627 (21.0)	685 (19.1)
10.4	843 (4.4)	840 (11.6)	649 (2.5)	673 (18.0)

Thus, BSF and Eosin displayed stable fluorescent readings over the pH range from 6.9–10.4. Pyranine was the most sensitive to pH changes among the five tested fluorescent substances, while Fluorescein and Tinopal were somewhat affected by the pH of the wash solution. However, Fluorescein, Pyranine, and Tinopal were more fluorescent sensitive than BSF and Eosin. Based on the results of this research, it required 0.024 µg/mL Fluorescein, 0.055 µg/mL Pyranine, 0.026 µg/mL Tinopal, 0.24 µg/mL Eosin, or 2.0 µg/mL BSF for the fluorescent intensity to reach 700 with the stable solution pH range (8.4 or higher). This result indicated that at a solution pH of 8.4 or higher, the fluorescent sensitivity of Fluorescein was 83 times that of BSF and 10 times that of Eosin, Tinopal was 77 times that of BSF and 9 times that of Eosin, and Pyranine was 36 times that of BSF and 4.4 times that of Eosin. It was necessary that the solution pH must be adjusted to be greater than 8.4 to reduce the analytical errors if Fluorescein, Pyranine, or Tinopal were used as the tracer to measure spray deposition and drift.

Conclusions

Fluorescent intensity of Pyranine, Fluorescein, and Tinopal increased as the solution pH increased within the range of 6.9–8.4, and then tended to become nearly constant for the solution pH beyond 8.4. The fluorescence of BSF and Eosin remained nearly constant over the solution pH range from 6.9–10.4.

Among the five fluorescent tracers tested, fluorescence of Pyranine was most affected by solution pH conditions, followed by Fluorescein and Tinopal. When the solution pH value increased from 6.9–8.4, the fluorescent intensity of solutions containing Pyranine, Fluorescein, and Tinopal increased 3.0, 1.25, and 1.2 times, respectively.

Pyranine, Fluorescein, and Tinopal had much higher fluorescent sensitivity than BSF and Eosin. To obtain the same level of fluorescent intensity at the solution pH 8.4, the amount of BSF should be 83 times that of Fluorescein, 77 times that Tinopal, and 36 times that of Pyranine. However, to minimize the error in the fluorescent intensity analysis, the pH value of solutions containing Pyranine, Fluorescein, and Tinopal should be adjusted to 8.4 or higher.

It is necessary to examine the fluorescence of tracers under various pH conditions during the selection of tracers for pesticide spray deposition and drift trials.

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